Supplemental Box and Table

Box 3: Strategies for Dynamin Inhibition

Inhibition of dynamin function is an important research tool to explore the action of this protein and the impact of endocytosis on a variety of cell processes. Methods to inhibit dynamin function (and their limitations in red) are listed below.

+ Temperature sensitive(^{ts}) mutations. Acute functional disruption, fully reversible. Dominant phenotype, possibly reflecting presence of mutant dynamin subunits in a dynamin polymer. (The dominant negative effect may also include sequestration by mutant dynamin of dynamin binding proteins and thus may reflect the disruption of the function of such proteins).

+ Transfection of mutant dynamin. Excess mutant dynamin results in a powerful dominant negative effect. (same limitations as for temperature sensitive mutations (see above); the indirect effect due to the sequestration of dynamin binding partners may be more robust due to mutant dynamin overexpression).

+ Micro-injection of anti-dynamin antibodies. Allows for acute functional disruption. (Blocked proteins may sequester interacting partners).

+ Microinjection of dynamin binding SH3 domains. Acute effects due to competition with endogeneous SH3 domain containing proteins for binding to dynamin's PRD.

(Many such SH3 domains also bind other proteins thus limiting the specificity of the method).

+ Microinjection of peptides from dynamin's PRD to outcompete endogenous dynamin for binding to physiological binding partners. (Since binding surfaces for dynamin's PRD, primarily SH3 domains, also bind other proteins, such peptides block these other interactions as well.)

+ Microinjection of GTPγS, a slowly hydrolysable analogue of GTP to lock dynamin in its GTP-bound conformation. (GTPγS will non-selectively inhibit other GTPases).

+ Pharmacological inhibition. Acute effect. (Most currently available drugs that impair dynamin GTPase activity function non-competitively and by unknown mechanisms. Off-target effects may occur. For example, the commonly used dynasore compound also inhibits at least some other DLPs [1].

+ RNAi-mediated knock-down. (Only partial elimination of function. Robust knock down of more than one dynamin isoform may be needed for effective loss of function).

+ Global and conditional gene KO. Can provide information on role of dynamin isoforms at the organismal level. (Negative impacts on development and viability complicate the use of these methods).

Table 2: Dynamin Interacting Proteins

Dynamin Interacting Protein	Mechanism of interaction	Reference
BAR Family		
Amphiphysins 1 and 2	Binding of dynamin PRD to SH3 domain	[2-6]
DNMBP1/Tuba, Cdc42 GEF activity	Binding of dynamin PRD to SH3 domains	[7, 8]
Endophilins 1, 2 and 3	Binding of dynamin PRD to SH3 domain	[9-14]
GRAF1	Binding of dynamin PRD to SH3 domain	[15]
SNX9, 18 and 30	Binding of dynamin PRD to SH3 domain	[16-19]
F-BAR Family		
Synadpins 1 and 2 (aka PACSINs)	Binding of dynamin PRD to SH3 domain	[20-23]
CIP4, FBP17, TOCA1	Binding of dynamin PRD to SH3 domain	[24, 25]
Nervous Wreck	Binding of dynamin PRD to SH3 domain	[26, 27]
Enzymes		
Calcineurin	PxIxIT motif in Dynamin 1b splice variant	[28-30] Giovedi, Ferguson and De Camilli unpublished observations.

c-Src, Tyrosine kinase	Binding of dynamin PRD to SH3 domain	[31, 32]
eNOS, nitric oxide synthase	Dynamin PRD-eNOS reductase domain	[33]
PI3K p85 subunit, Lipid kinase	Binding of dynamin PRD to SH3 domain	[31, 34]
PLCγ, Phospholipase activity	Binding of dynamin PRD to SH3 domain	[35, 36]
Signaling **Adaptors/Scaffolds**		
Arc	Via dynamin PH domain	[37]
CIN85 and CD2AP	Binding of dynamin PRD to SH3 domain	[38, 39] and Ferguson and De Camilli unpublished data.
Grb2	Binding of dynamin PRD to SH3 domain	[31, 35, 36, 40, 41]
Homer 1	PxxF motif in Dynamin 3 PRD interacts with EVH1 domain	[42-44]
Intersectin 1, Cdc42 GEF activity (splice variant)	Binding of dynamin PRD to SH3 domains	[14, 45-48]
Nck	Binding of dynamin PRD to SH3 domain	[49, 50]
Nef,	Binds selectively to the dynamin 2	[51]

HIV protein	isoform via the middle and GED domains	
SPIN90/WISH	Binding of dynamin PRD to SH3 domain	[52]
TTP	Binding of dynamin PRD to SH3 domain	[53]
Vav1, Rho GEF activity	Binding of dynamin PRD to SH3 domain	[54]
Cytoskeleton		
ABP1, Binds F-actin	Binding of dynamin PRD to SH3 domain	[55]
Cortactin, Binds F-actin	Binding of dynamin PRD to SH3 domain	[56-58]
F-Actin	Actin filaments bound via middle domain	[59]
Kalirin 12, Rho GEF activity	IgFn domain interaction with dynamin GTPase domain	[60]
Myosin 1E, Actin based motor	SH3 domain-PRD interaction	[61]
Microtubules	Via dynamin PRD	[62-64]
γ-tubulin	Via middle domain	[65]

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